THE ORIGIN AND BEHAVIOUR OF CHIASMATA

V. Chorthippus elegans

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Introduction

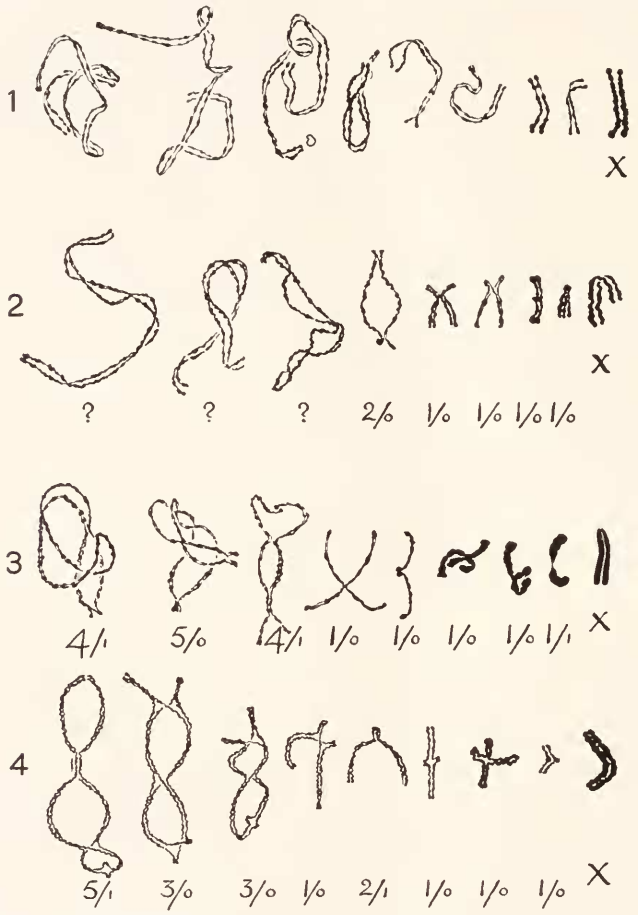
At the pachytene stage of the prophase of meiosis the chromosomes are single threads and these threads lie side by side in pairs throughout their length: association is complete. But at the end of this stage the threads fall apart; they appear to repel one another and they are found to be longitudinally double, owing to the division of each chromosome into two "chromatids." The double threads remain associated, however, in spite of their repulsion, for the chromatids change partners at one or more places which are known as "chiasmata." The chiasmata persist with various changes in number and position from this diplotene stage until metaphase and they are important, first, on account of their function in holding the chromosomes together and allowing them to continue paired until metaphase, and, secondly, on account of their being the result of genetic crossing over between chromatids of opposite chromosomes (*cf.* Darlington, 1932). In order to understand their cause and function properly it is necessary to record their varying numbers and positions at successive stages of prophase in large numbers of organisms and so deduce their behaviour and the forces determining their origin and maintenance.

These forces have very different effects in different organisms and the subject of the present study, *Chorthippus elegans* (Acrididæ) is an example of a species with the least possible change in the number and position of the chiasmata between diplotene and metaphase. The methods used were the same as in *Stenobothrus* (Darlington and Dark, 1932) except that all the observations are from one smear preparation of one individual (giving the maximum uniformity of material). This was stained with Heidenhain's hæmatoxylin instead of gentian violet; the result, though less satisfactory for tracing the course of the separate chromatids at diplotene, is relatively free from danger of fading.

Observations of Spermatogenesis

Pachytene.—Eight double threads, the paired autosomes, can be distinguished together with one more condensed and homogeneous body,

the X chromosome. No doubleness can be definitely made out in the autosome threads until the end of this stage, but the upturned ends of chromosomes show what may be the beginnings of a split. The X



FIGS. 1–12. The eight paired autosomes and the X chromosome at successive stages of prophase. The total number of chiasmata and the number terminal are given under each bivalent in the later stages. × ca. 2000.

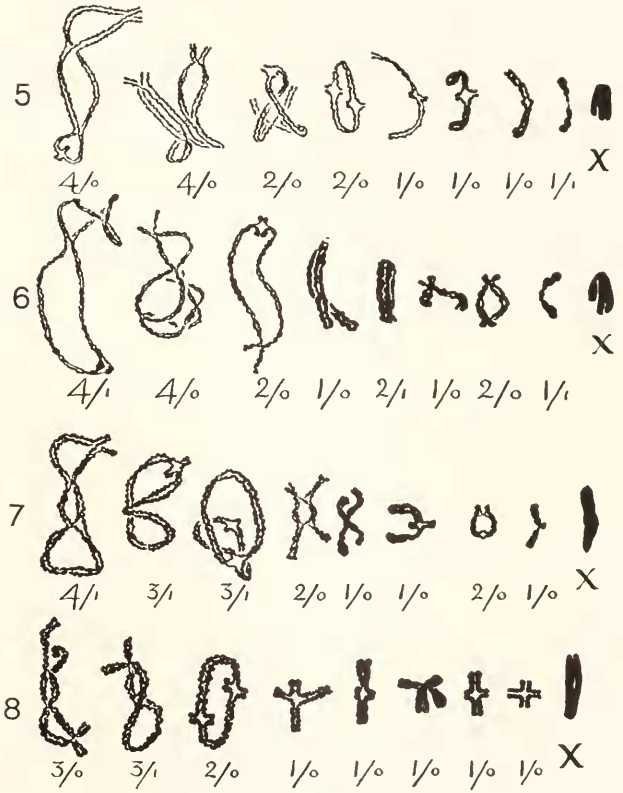
FIG. 1. The end of pachytene.

FIG. 2. The beginning of diplotene. Separation of the shorter chromosomes complete.

FIGS. 3 and 4. All loops completely open and chiasmata recognizable.

chromosome, however, has already divided into two halves. There is little evidence of a "bouquet" stage at zygotene and none persists through pachytene.

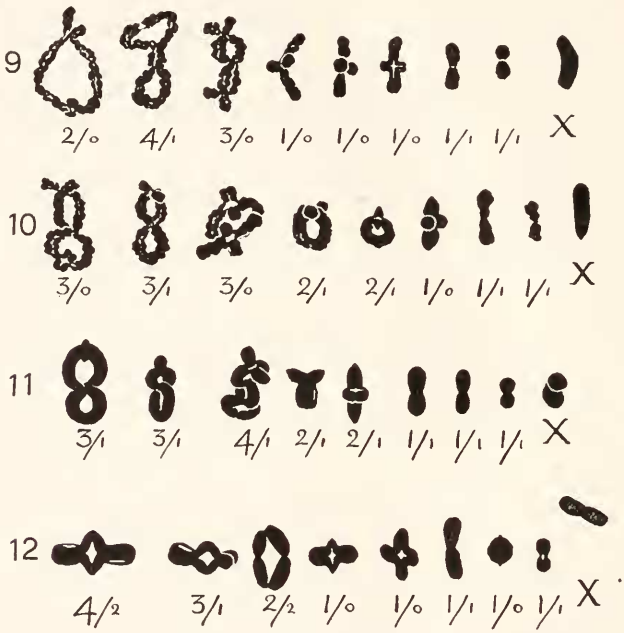
Diplotene to Diakinesis.—The paired threads fall apart (Fig. 1). The long chromosomes with median constrictions begin to separate either in the middle or at one end. Doubleness is still not evident.[1] The separation is first complete in the short chromosomes (Fig. 2). These are also found to be slightly in advance of the longer ones in condensation (Fig. 3) and they maintain this difference until diakinesis. This suggests that differential precocity ("heteropycnosis") here de-



FIGS. 5–8. Successive stages of diplotene.

[1] Robertson and others have stated that the chromosomes are double before they pair but do not claim to have any direct evidence to show that this is so. The *indirect* evidence they bring forward consists in the well-known supposition of "anaphase duality" which is now seen to depend on a misinterpretation of spiral structure (Darlington, 1932). On the other hand, all *direct* observers of zygotene in plants and animals agree that the chromosomes are single at this stage (Gelei, Wenrich, Belling, Newton and Darlington). Speculations as to the possible earlier division of the chromosomes at meiosis and mitosis conflict with the precocity theory of meiosis (Darlington, 1931) but are not valid evidence against it.

pends on size differences. The loops first meet, revealing the number and position of the chiasmata, in the shorter pairs. As the loops open the doubleness of the chromosomes becomes detectable (Fig. 2). The chromatids can then be followed separately through the chiasmata and sometimes throughout their length. Later they swell considerably and cease to be separately identifiable, except at the chiasmata (Fig. 9). This swelling is seen in many animals (perhaps *Pristiurus* may be regarded as the extreme type) and also in a gymnosperm, *Taxus baccata*



Figs. 9 and 10. Diakinesis.
Fig. 11. Pro-metaphase.
Fig. 12. Metaphase, X chromosome lying to one side of the spindle. *Note:* the three long pairs of chromosomes have median spindle attachments, the rest terminal.

(Dark, unpublished). It is characteristically different from the behaviour in the angiosperms where the more swollen condition prevails throughout the post-pachytene stages and seems to prevent the chromatids being so clearly distinguished at diplotene as in some Orthoptera.

The differences between angiosperm and orthopteran may, of course, be artefacts but it is impossible to say which is normal and which the artefact. Many would infer that the clearer observation is more true-to-life but such a conclusion does not necessarily follow. The increase in size has a curious effect. Owing to the differential but somewhat

variable rate of condensation of short and long chromosomes, their relative sizes during the diplotene phase change rather suddenly and interfere with the constant distinction of types (Figs. 3–6).

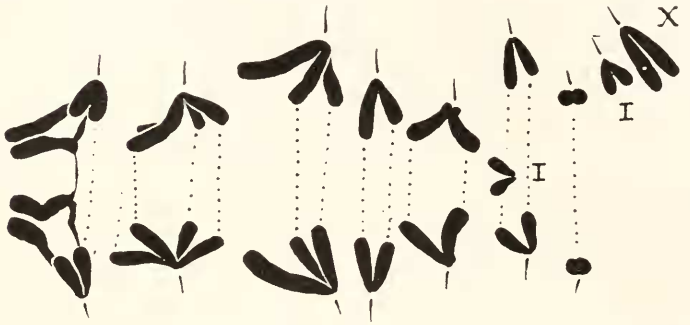


Fig. 13. Anaphase of the first division following failure of pairing of the shortest medium pair; one of the univalents is about to divide at the first division. The chromatids that have been associated are connected by a dotted line. × ca. 3000.

Figs. 14 and 15. Second division. × ca. 3000.

Fig. 14. Polar view of the eight autosomes in metaphase.

Fig. 15. The two divisions of one spermatocyte. Above, anaphase; nine chromosomes including the X chromosome. Below, metaphase (chromosomes drawn separately); the distal ends of some of the chromosomes are coming together.

Metaphase to Anaphase.—Between pachytene and metaphase the chromosomes contract to about one-sixth their length. The eight bivalents lie on the equatorial plate and the X chromosomes to one side

of it (Fig. 12). Repelling one another from their spindle attachments the pairs of chromatids associated at these points pass to opposite poles. Those pairs distal to the first chiasma therefore have to separate and the strain often draws them into a fine thread. Exceptionally a connection is seen between the separating chromatids (Fig. 13), as already noticed in *Hyacinthus* and *Stenobothrus*. This connection is as yet unexplained. Exceptionally also two of the shorter chromosomes are found to be unpaired and may lag and divide at the first division. This failure corresponds with observations during prophase and, as will be seen, is related to conditions of chiasma formation.

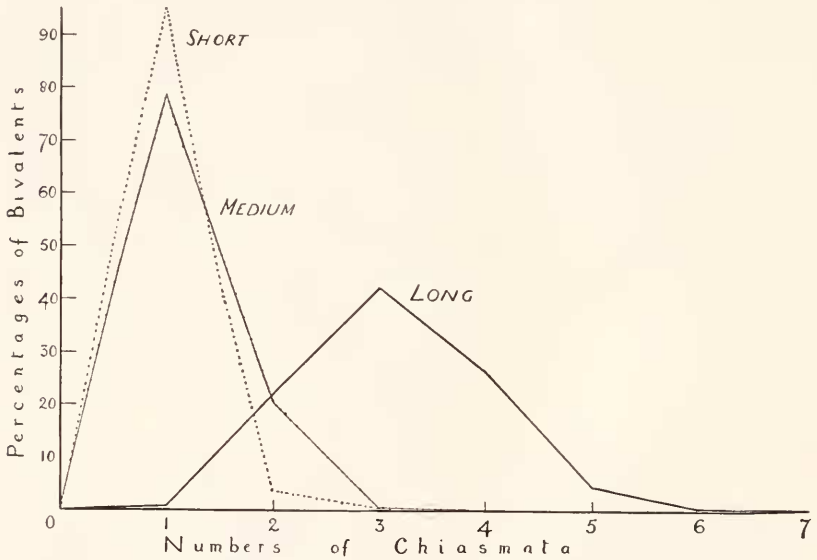


Fig. 16. Graph showing variation in chiasma frequency in the long, medium, and short types of chromosome.

Second Division.—During the interphase and early metaphase of the second division the pairs of chromatids are held together only at their spindle attachments (Fig. 14). They suddenly come together, immediately before anaphase, it must be presumed, for they are rarely seen together during metaphase and then only touching at the ends (Fig. 15).

Syndiploidy.—Groups of from two to six spermatocyte nuclei are often found closely appressed, between pachytene and diakinesis; and at metaphase, in a corresponding proportion of cells, fusion of the adjoining plates of chromosomes is seen. Where only two nuclei lie together the result is a regular fused spindle but, with a larger number, the chromosomes no longer orientate themselves regularly. These re-

sults are closely paralleled by Eisentraut's observations (1926) on *Gomphocerus maculatus* and by numerous observations of syndiploidy in plants, although the exact time of fusion, the onset of metaphase, is not elsewhere so clear. It is evident that in all these cases fusion takes place after pachytene and, although four homologous chromosomes of each type are present (in both plants and animals), quadrivalents are

TABLE I

Summary of Observations of 100 Nuclei (800 Bivalents)

Numbers of Terminal Chiasmata in each Bivalent		Numbers of Chiasmata per Bivalent											
		long type (three)						medium type (four)				short type (one)	
		1	2	3	4	5	6	0	1	2	3	1	2
Mid Diplotene (24 nuclei)	0	—	11	20	9	4	—	—	67	11	—	8	—
	1	1	3	9	7	2	—	—	3	15	—	14	2
	2	—	1	2	1	1	1	—	—	—	—	—	—
Total		1	15	31	17	7	1	—	70	26	—	22	2
Late Diplotene (16 nuclei)	0	—	8	10	4	—	—	—	41	7	—	3	—
	1	—	3	13	7	—	—	—	10	6	—	13	—
	2	—	—	1	—	2	—	—	—	—	—	—	—
Total		—	11	24	11	2	—	—	51	13	—	16	—
Diakinesis (40 nuclei)	0	2	14	17	2	1	—	1	90	—	—	4	—
	1	—	12	30	15	2	—	—	43	25	1	35	1
	2	—	1	12	10	2	—	—	—	—	—	—	—
Total		2	27	59	27	5	—	1	133	25	1	39	1
Metaphase (20 nuclei)	0	—	3	3	1	—	—	—	31	—	—	4	—
	1	—	9	12	9	—	—	—	30	18	1	15	1
	2	—	1	8	14	—	—	—	—	—	—	—	—
Total		—	13	23	24	—	—	—	61	18	1	19	1
Grand Total		3	66	137	79	14	1	1	315	82	2	96	4
Percentages		1	22	45.7	26.3	4.7	0.3	0.3	78.7	20.5	0.5	96	4

never formed. Evidently, therefore, the pairing of chromosomes at metaphase in these organisms can only be derived from pachytene pairing and does not arise from a direct affinity. This conclusion follows from the chiasma theory of pairing and contradicts the assumption that the chromosomes are paired at metaphase on account of any attraction operating between them at this stage (Darlington, 1931).

Chiasma Frequency.—The numbers of chiasmata present in the bivalents of each type remain without significant change from diplotene to metaphase. They show (Fig. 16) the interference curves of frequency variation constantly observed in all organisms so far studied (Haldane, 1931; *cf.* Darlington, 1932). They also show (Table II) the indirect relationship of length of chromosome to frequency already found in *Stenobothrus* and probably very general in organisms with

TABLE II

Mean Chiasma Frequencies per Bivalent (derived from Table I)

		Mid Diplotene Stage (24 nuclei)	Late Diplotene Stage (16 nuclei)	Diakinesis (40 nuclei)	Metaphase (20 nuclei)	Total (100 nuclei)	Observations on Stenobothrus parallelus	Length
Long Type	No. Bivalents...	72	48	120	60	300		11.3 μ
	Total No. Chiasmata.....	233	148	366	191	938		
	No. Terminal Chiasmata....	34	29	109	76	—		
	Chiasma Frequency per Bivalent.....	3.24	3.08	3.05	3.18	3.13	3.31	
	Terminal Chiasmata per Bivalent.....	.47	.60	.91	1.27	—		
Medium Type	No. Bivalents...	96	64	160	80	400		4.1 μ
	Total No. Chiasmata.....	122	77	186	100	485		
	No. Terminal Chiasmata....	18	16	69	49	—		
	Chiasma Frequency per Bivalent.....	1.27	1.20	1.16	1.25	1.21	1.45	
	Terminal Chiasmata per Bivalent.....	.18	.25	.43	.61	—		
Short Type	No. Bivalents...	24	16	40	20	100		1.6 μ
	Total No. Chiasmata.....	26	16	41	21	104		
	No. Terminal Chiasmata....	16	13	36	16	—		
	Chiasma Frequency per Bivalent.....	1.08	1.00	1.02	1.05	1.04	1.04	
	Terminal Chiasmata per Bivalent.....	.61	.81	.90	.80	—		

found in *Stenobothrus* and probably very general in organisms with

wide range of size amongst the chromosomes (Darlington and Dark, 1932). This effect is not so pronounced, however, in the medium chromosomes as in *Stenobothrus* with the predictable result (on the chiasma theory of pairing) that the shortest member of this type occasionally fails to pair (Fig. 13, anaphase, and Table I, diakinesis).

Terminalization.—The total number of chiasmata remains the same, but the proportion of these that are terminal increases during prophase and it increases in a characteristically different way in the different types of bivalent and in those with different total numbers of chiasmata (Table III and Fig. 17). This agrees with the assumption that the terminal chiasmata arise by movement of earlier interstitial ones to the ends. Thus, amongst those with 2, 3, and 4 chiasmata, the proportion that are terminal (the *terminalization coefficient*) is the same at each stage. This indicates that originally all the chiasmata were interstitial as required by the hypothesis. Further, as in *Tulipa* (Darlington and Janaki

TABLE III

Numbers of terminal chiasmata per bivalent in bivalents with different total numbers of chiasmata (derived from Table I)

Stage	Long Type			Medium Type		Short Type
	2	3	4	1	2	1
Mid Diplotene	.33	.42	.53	.04	.58	.64
Late Diplotene	.27	.62	.64	.20	.46	.81
Diakinesis	.52	.91	1.30	.48	1.00	.90
Metaphase	.84	1.22	1.54	.49	1.00	.79

Ammal, 1932), those with one chiasma show less movement, especially in the early stages, than those with two or more. Finally, the occurrence of more terminal chiasmata at the same stages in the corresponding classes of the shorter chromosomes is to be expected on the assumption of movement since the chiasmata are more concentrated in these chromosomes and have not so far to go to reach the ends.

The only change that takes place in terminalization in the long chromosomes in this organism is the expansion of closed loops at the expense of the distal arms, a change which according to the electrostatic hypothesis (Darlington and Dark, 1932) is due to a repulsion between distributed surface charges on the paired chromatids. In the medium and short chromosomes there is also a slight movement of single chiasmata owing to the special repulsion of the spindle attachments, but this movement is ineffective in moving a proximal chiasma against the repulsion of a loop when two are present owing to the terminal spindle attachments always lying in open arms in which repulsion is less effective.

The terminalization of the chiasmata in the short chromosomes is therefore very like that in the fragments of *Fritillaria imperialis* where all chiasmata are terminalized early although only the distal chiasmata move to the end in the major chromosomes with numerous chiasmata (Darlington, 1930).

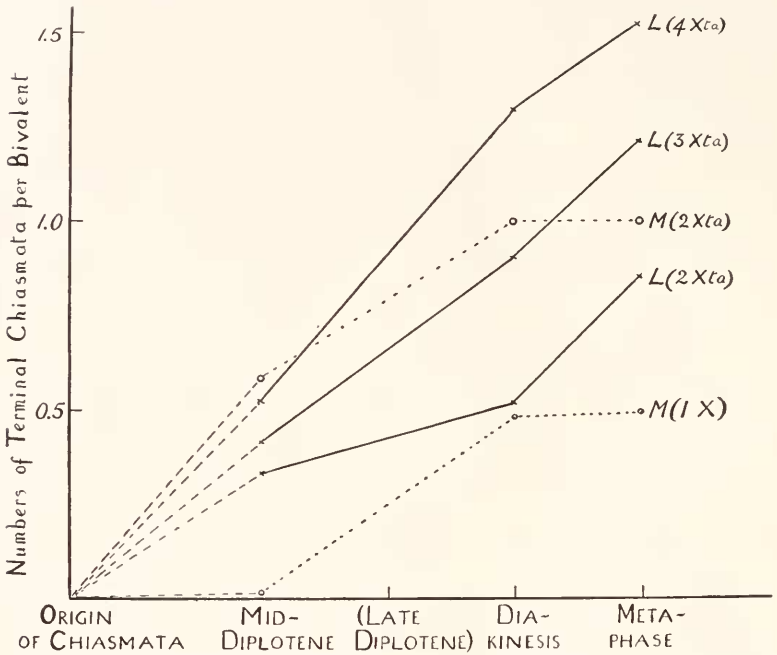


FIG. 17. Graph showing that the increase in the numbers of terminal chiasmata is in proportion to the total number at all stages where more than two are formed but not in the earlier stages where only one is formed. The short chromosome type and the late diplotene stage are omitted because the numbers of observations are smaller than those given.

SUMMARY

1. A study of meiosis in male *Chorthippus elegans,* Acrididæ ($2n = 16 + X$) shows the chromosome behaviour to be similar to that already described in *Stenobothrus parallelus.* Thus the chiasma frequency is an indirect function of length and has an interference curve of variation. Failure of pairing occurs in one chromosome type with a frequency in keeping with the curve. Terminalization depends entirely on the generalized repulsion in bivalents with closed loops and the localized spindle attachment repulsions are of the minimum degree found in *Fritillaria.*

2. A more extensive quantitative study makes it possible to show in the long chromosome type, that the number of terminal chiasmata at each stage between diplotene and metaphase is proportional to the number of interstitial chiasmata and increases from one stage to the next *pari passu* with the decrease in the number of the interstitial chiasmata. This confirms the earlier arguments that all interstitial chiasmata become terminal by movement without breakage while, on the other hand, terminal chiasmata always arise from earlier interstitial ones and in no other way. It is now clear that chiasmata always change their position after their formation at diplotene so that the configurations observed later are merely positions of changing equilibrium.

3. The opening of the diplotene loops has been followed in detail and shows that the chromosomes are single and undivided until this stage. The opening is therefore derived solely from the so-called " reductional " split, not from the " equational " one which only begins to appear at this time.

4. Syndiploidy occurs frequently just before metaphase.

For bibliography, see p. 370 of the following paper by the same author.